Nutritional analysis of oleaginous algae from different reservoirs of Haryana and enhancement of its lipid and EPA yield

न्यूट्रिशनलएनालिसिसऑफ़ओलिऐजिनॅसएलगीफ्रॉमडिफरेंटरेज़र्वोयरजऑफ़हरियाणाएंडएनहांसमेंटऑफ़इट्सलिपिडएंडईपीएयील्ड

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Amanjyoti

Department of Food Science and Technology

Chaudhay Devi Lal University

Sirsa, Haryana-125055

Detailed synopsis for registration to Ph.D. program in Food Science and Technology

Proposed title of Ph.D. thesis: Nutritional analysis of oleaginous algae from different reservoirs of Haryana and enhancement of its lipid and EPA yield

Name of candidate: Amanjyoti

Supervisor: Dr. Manju V. Nehra

Assistant Professor and Incharge,

Department of Food Science and Technology,

Chaudhary Devi Lal University, Sirsa-125055

Introduction

Microalgae are single-celled and in the form of microscopic plants are called "phytoplankton". Microalgae have the ability to synthetize many compounds, some of which have been recognized as a source of functional ingredients for nutraceuticals with positive health effects. Promya *et al.,* (2008) reported that algae are significant source of human food, especially in Asia and cultivated for nutrition and pigments for supplemental use as human food and animal feed. Nutritional value of *Cladophora* show that it contains significant amounts of protein and carotenoids, which are essential for human and fish nutrition (Khuantrairong and Traichaiyaporn, 2009; Traichaiyaporn *et al*., 2010).

Epifanio, (1979) studied that the nutritional value of a microalgal diet contains both essential macro- and micronutrients to the target animal consumer. Microalgae provide many phytonutrients, including in particular PUFAs e.g.Eicosapentaenic acid(EPA), arachidonic acid (AA) and docosahexaenoic acid (DHA), which are known to be essential for various marine animals (Nichols, 2003). Microalgae contain 30-40% protein and 10-20% lipids in the late-logarithmic growth phase (Renaud *et al*., 1999 and Brown, 1997). Lipid membranes contain sterols such as fucosterol and β-sitosterol (Fahy *et al.,* 2005) that also have reported health benefits (Arul*etal.,* 2012). Most microalgal species contain 7-34% EPA and 0.2-11% DHA (Brown.1997).

The brain is a structural-lipid richorgan that uses highly unsaturated fatty acids, particularly AA and DHA, for structure and function (Crawford *et al.,* 1997; Crawford and Sinclair, 1972). It is usual to consider that AA and DHA are synthesized from their parent precursors, linoleic (LA; 18:2n26) and alph-linolenic (ALA; 18:3n23) acids.

Eicosapentaenic acid (EPA, 20:5 ω3) and docosahexaenoic acid (DHA, 22:6 ω3) are the important omega-3-PUFA, while arachidonic acid (AA, 20:4 ω6), is a vital omega-6-PUFA. EPA and DHA are important in treatment of arthrosclerosis, cancer, rheumatoid arthritis, psoriasis and diseases of old age such as Alzheimer's and age-related mascular degeneration (Drevon *et al.,* 1993; Simopoulos *et al.,*1999). AA and DHA are of special importance in the brain and blood vessels, and are considered essential for pre- and post-natal brain and retinal development (Crawford, 2000). The eicosanoids, such as prostaglandins, prostacyclins and leukotrienes, derived from omega-3-PUFA are also important in new-born and infant development, modulatory vascular resistance and wound healing (Simopoulos *et al.,*1999; Nettleton, 1995).Microalgae are prized for their micronutritional value, PUFA, containing

immuno-stimulant substances, fight cancer, protect us from UV radiation and treat joint ailments, to mention but a few applications. The removal of heavy metals from polluted soil and water and some have even tried for biological CO_2 fixation in fuel gas of industrial plants (Moore, 2001).

The PUFAs 20:5(n3) and 22:6(n-3) are essential fatty acids (EFAs) for the survival and growth of many juvenille aquaculture organisms (Langdon and Walcock, 1981; Levine andSulkin, 1984; Enright *et al.,* 1986).The importance of polyunsaturated fatty acids (PUFA) in human nutrition and disease prevention was scientically recognized three decades ago. The long-chain PUFA, namely eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), may be acquired mainly from seafoods or derived from -linolenic acid by a series of chain elongation and desaturation (Sprecher, 1995). EPA and DHA are synthesized mainly by both uni and multicellular marine plants such as phytoplankton and algae (Pigott and Tucker, 1987).

Among algae of alpine environment there could be strains particularly rich in long chain polyunsaturated fatty acids (LC-PUFA). Indeed, the *chlorophyte* (*Trebuxiophyceae*) *Parietochloris incisa* isolated from *Mt. Tateyama*, Japan, was found to be the richest plant source of the pharmaceutically valuable LC-PUFA, arachidonic acid (AA, 20:4ω 6). The algae are also extremely rich in triacylglycerols (TAG), which reaches 43% (of total fatty acids) in the logarithmic phase and up to 77% in the stationary phase. In contrast to most algae whose TAG are made of mainly saturated and monounsaturated fatty acids, TAG of *P. incisa* are the major lipid class where AA is deposited, reaching up to 47% in the stationary phase. Except for the presence of AA, the PUFA composition of the chloroplastic lipids resembled that of green algae, consisting predominantly of C_{16} and C_{18} PUFAs. The composition of the extrachloroplastic lipids is rare, including phosphatidylcholine (PC), phosphatidylethanolamine (PE) as well as diacylglyceryltrimethylhomoserine (DGTS). PC and PE are particularly rich in AA and are also the major depots of the presumed precursors of AA, l8:36 and 20:3 ω 6, respectively. Long-chain polyunsaturated fatty acids (LC-PUFAs) of the omega-3 family are quite abundant in microalgae. For example, *Porphyridiumcruentum* (Cohen *et al.,* 1988), *Nannochloropsis sp.* (Seto *et al.,* 1984; Sukenik and Carmeli, 1989), *Phaeodactylum tricornutum* (Yongmanitchai and Ward, 1992; Molina Grima *et al.,* 1999) and *Monodus subterraneus* (Cohen, 1994) were studied for their potential to produce eicosapentaenoic acid (EPA, 20:5ω 3). Likewise, *Crypthecodinium cohnii* (Jiang *et al.,* 1999) and *Chroomonas salina* (Henderson and MacKinlay, 1992) contain docosahexaenoic acid

(DHA, 22:6ω 3). However, omega-6-LC-PUFAs are relatively rare. High contents of 20:3 ω 6 were not found in any organism. Arachidonic acid (AA, 20:4 ω 6) is almost excluded from the lipids of fresh water algae and in most marine species it does not account for more than a few per cent of total fatty acids (Thompson, 1996). The only microalga reported to produce AA in significant quantities is the unicellular rhodophyte *P. cruentum* (Cohen, 1990). Under logarithmic growth, the major PUFA of this alga is EPA, but under unfavorable conditions, AA predominated, reaching 40% of total fatty acids. In other rhodophytes such as *Gracilaria sp.*, the proportion of AA can be as high as 60% of total fatty acids; however, the dry weight content does not exceed 0.2% (Araki *et al.*, 1990). When present, LC-PUFAs are predominantly located in the polar membranal lipids, whereas triacylglycerols (TAG) generally contain very little PUFAs (Cohen, 1999). The capacity for AA accumulation makes *P. incisa* one of the best candidates for large-scale production of AA. This PUFA was shown to be a major component of brain cell membranes, as well as of breast milk (Hansen *et al.*, 1997). It was thus suggested that the diet of preterm infants that are not breast-fed should be implemented with AA by supplementation of newborn milk formula (Carlson *et al.*, 1993; Boswell *et al.*, 1996). The finding that most AA of *P. incisa* is deposited in TAG is of practical value since TAG are the preferred chemical form for the introduction of AA into baby formulated foods.

Objectives:

1. Screening of oleaginous algae from different reservoirs of Haryana.
2. Effect of mixotrophic growth conditions on yield of oil from selected microalgae.
3. Effect of phytohormone treatment on EPA yield of selected microalgae.
4. Generation of mutants and their screening for high lipid and EPA yield.

Review

Patil *et al.*,(2005) reported that Microalgae as source of polyunsaturated fatty acids for aquaculture.The therapeutic significance of polyunsaturated fatty acid (PUFA) especially docosahexaenic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) has been demonstrated by recent clinical and epidemiological studies. Fish oils are the major commercial source of long chained omega-3-PUFA. Global production of farmed fish and shell fish has more than doubled in the past two decades, trends toward intensification and greater control over nutritional input resulting in increased demand for wild fish for feed. Feed is the largest production cost for commercial aquaculture (e.g. most farming of salmon, other marine finfish, and shrimp), and thus improving feed efficiency in industrial systems is already a priority. Moreover, fishmeal prices have risen in real terms in the past three decades and are likely to increase further with continued growth in demand. The possible decline of commercial fish stocks calls for research in alternative sources of PUFA. Considerable evidence has indicated that omega-3-fatty acids in fish oils actually derive from zooplankton that consumes algae. Further the microalgae may have superior lipid stability compared to traditional PUFA because they are naturally rich in antioxidant carotenoids and vitamins and because lipids are microencapsulated by the algae cell wall.

Peltomaa *et al.*,(2017) studied that microalgae have the ability to synthetize many compounds, some of which have been recognized as a source of functional ingredients for nutraceuticals with positive health effects. One well-known example is the long-chain polyunsaturated fatty acids (PUFAs), which are essential for human nutrition. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the two mostimportantlong-chainomega-3-PUFAs involved in human physiology, and both industries are almost exclusively based on microalgae. In addition, algae produce phytosterols that reduce serum cholesterol. Here they determined the growth rates, biomass yields, PUFA and sterol content, and daily gain of eight strains of marine cryptophytes. The maximal growth rates of the cryptophytes varied between 0.34–0.70 divisions day−1, which is relatively good in relation to previously screened algal taxa. The studied cryptophytes were extremely rich in ω-3 PUFAs, especially in EPA and DHA (range 5.8–12.5 and 0.8–6.1 μg mg dry weight−1, respectively), but their sterol concentrations were low. Among the studied strains, *Storeatula* major was superior in PUFA production, and it also produces all PUFAs, i.e., α-linolenic acid (ALA), stearidonic acid (SDA), EPA, and DHA, which is rare in phytoplankton in general.

They conclude that marine cryptophytes are a good alternative for the ecologically sustainable and profitable production of health-promoting lipids.

Kitano*et al.*,(1997) evaluated that change in eicosapentaenoic acid content of *Navicula saprophila, Rhodomonas salina* and *Nitzschia sp.* under mixotrophic conditions. Three species of microalgae able to produce eicosapentaenoic acid (EPA) were collected from brackish and sea water around Japan. The species were identified as *Navicula saprophila, Rhodomonassalina* and *Nitzschia sp*. EPA as a proportion of total fatty acids increased in the presence of acetic acid for *Rhodomonas salina* and *Nitzschia sp.* However, *Navicula saprophila* displayed the greatest productivity of EPA and the EPA content of its biomass was enhanced under mixotrophic conditions in the presence of acetic acid.

Volkman *et al.*,(1989) reported that total fatty-acid composition, lipid classes, Chl a contents and ceil volumes of 10 species of microalgae commonly used in mariculture were examined. The microalgae were grown under defined conditions and harvested during log phase. Significant concentrations of the polyunsaturated fatty acid 20: 5(n-3) (eicosapentaenoic acid) were present in each of the four diatoms *Chaetoceros calcitrans, Chaetocerosgracilis, Skeletonema costatum* and *Thalassiosirapseudonana* (4.6 - 11.1% of the total fatty acids), the *cryptomonad Chroomonas sahna* (10.9, 11.9%) and the prymnesiophyte*Pavlova lutheri*(19.7%). Very small amounts were found in another *prymnesiophyte, Isochrysis sp.*, and the green algae *Nannochloris atomus* and *Tetraselmis suecica* but none was detected in the green alga *Dunaliella tertitolecta*. In contrast, high concentrations of 22: 6(n-3) (decosohexaenoic acid) were present only in the two prymnesiophyte algae and *Chroomonas salina*. Chl a and total fatty-acid contents were not related to cell volume. Fatty acids were four to six times more abundant than Chl a in most species. Relative concentrations of intact lipids were determined for each species using Iatroscan thin-layer chromatography-flame-ionisation detection. Polar lipids predominated in all species but triacyglycerols were abundant only in *Chaetoceros gracilis, Isochrysis sp.* and *Chroomonas sp.* Free fatty acids were particularly abundant in the two *Chaetoceros* species. The importance of these data for determining the nutritional value of the microalgae as food for animals in mariculture is discussed.

Patil *et al.,* (2007) reported that twelve algal strains representing the classes *Cyanophyceae, Prymnesiophyceae, Bacillariophyceae, Rhodophyceae, Cryptophyceae, Chlorophyceae, Xantophyceae* and *Eustigmatophyceae* were selected mainly from the culture collection of the

Norwegian Institute for Water Research (NIVA). The algae were grown as continuous cultures in a 1.8 l. reactor, internally illuminated with an 11 W fluorescent tube. The retention time was adjusted in the range 2–4 days to fit the growth rate of the algae. The growth responses and fatty acid composition were analysed. The maximum production rate was obtained with *Pseudokirchneriella subcapitata* (0.63 g 1–1 day–1) and the lowest with *Porphyridium cruentum* 0.13 g 1–1 day–1. Arachidonic acid (AA) and eicosapentaenoic acid (EPA) were the dominating polyunsaturated fatty acids (PUFAs) in *P.cruentum,*while only EPA accumulated in *Phaeodactylumtricornutum*. Docosahexaenoic acid (DHA) was the major PUFA in *Isochrysis galbana*, while *Pavlovasp*. had both EPA and DHA. This is the first report on the fatty acid profiles of *Nannochloropsis oceanica, Chroococcus sp., Synechococcus sp.* and *Tribonema sp.*

Wu *et al.,* 2015 reported that the total lipid contents of the strains ranged from 22% to 33%. The LC (%) of *M. dybowskii*XJ-151 was the highest (33.5 ± 4.38), followed by *M. dybowskii* XJ-435 (33.2 ± 0.57), which was higher than the literature data for *M. dybowskii*. The overall FA compositions were similar, where the main components of FA were C_{16} and C_{18}. The contents of C_{16} to C_{18} were 86% to 98%, whereas the longchain FAs above C_{20} exhibited low quantities. By comparison, SFAs of *M. dybowskii* XJ-435 and *D. intermedius* XJ-99 showed the highest proportion (41.09% and 41.94%, respectively). Polyunsaturated fatty acid (PUFA) contents among them were different; the highest was 37.95% (*D. intermedius* XJ498) and the lowest was only 9.81% (*D. intermedius* XJ-99*). D. intermedius* XJ-498exhibited the lowest C18:1 content (18.15%), but had the highest C18:3 content (18.18%). However, C_{18} FA contents composed of PUFAs in algal oils were much less than in vegetable oils.

Methodology:

Various analytical techniques may be used to identify, isolate and extraction of algae as well as different components such as:

1) Screening: Screening will be determined by suitable method/s.
2) Culture plating: Culture plating will be determined by standard method/s.
3) FAMEs analysis via gas chromatography: FAMEs method will be determined by suitable method/s.
4) Statistical analysis: Data obtained though experimentation will be statistically analyzed by using suitable method/s.

   More methods may be increased/decreased as per requirement of the research work.

Significance of the study

1) Microalgae are used as food: They are rich in Protein, Vitamin, Polyunsaturated fatty acid and Minerals. e.g *Chorella* (high protein and lipid content) and *porphyra* (soups, salads and vegetables).
2) Microalgae are commercially available.
3) Nutritional deficiencies in a diet can be avoided by the use of mixed algae diets.
4) The future use of EPA for treatment of various diseases/ disorders would require extensive clinical trials.

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